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Enzymes: an Essay on
THE MIRACLE OF ENZYMES

The Power of Enzymes in Making Life.

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ABSTRACT

This essay builds on the earlier insight that proteins are the key molecules in building **LIFE** from organic chemistry. This related investigation goes **deeper** in **how** proteins achieve their **Magic** in the **physiology** of life by tracking what they do as enzymes. This completes the Life-Review extending amino acids, through proteins and now up to enzymes; on the road to understanding the living processes shown in cells and organs. This essay on enzymes was motivated to show some of the vast **complexity** introduced by enzymes and how little is known about this vast Jungle of Unknowns, so as to alert the general reader on the degree of ignorance still facing biological researchers, as they wildly rush into the unknown with all its hidden surprises still there. If you want to understand **LIFE** then you must learn something about **enzymes**.

Philosophers have made a huge mistake by seeing the world in terms of simple objects producing the theories of mathematics and physics, when it is **biology** that shows how it is the **relationships** (and interactions) can result in the **processes** of living cells. To understand **LIFE**, one must understand Enzymes, as they **interact** to transform simpler molecules into more complex forms. Since DNA is a linear view of simpler segments of proteins, it is only in their full three dimensional glory do we see them fully active. However, one cannot easily go from a linear static view of structures to the complexity of biochemical transformations needed to maintain life. We are cataloguing thousands of proteins as linear structures but it only when we understand the 3D activities of enzymes can we begin to understand life itself. As we have tried to show here, enzymes are vital to life but are also phenomenally **complicated**. Our mad rush to exploit science has resulted in our making deadly nerve gases that are so powerful, they qualify as "Weapons of Mass Destruction" (WMD).

Although written for an educated audience (but not biochemists) there is much here of interest to non-scientists to show HOW science is done (slowly: one discovery at a time). I hope this great story will appeal to many.

NB This essay contains valuable, **life saving** information on Blood Circulation Problems on page 15.

AUTHOR BIOGRAPHY

Dr. Herb Spencer (born in Glasgow, Scotland) is a writer, researcher and natural philosopher. He was trained as a theoretical physicist at **Imperial College** (London University) before losing interest in its too mathematical style after a short, successful post-doctoral period of publishing several papers in the leading physics journals based on his two-year PhD research thesis. He 'slipped' into the new world of computing and quickly built an understanding that allowed him to pursue a career in management-consulting about computer innovation in business. Eventually, he set up a new software company to offer fixed-price projects for several industries; finally writing a gigantic software system for the **insurance** industry. This provided him with sufficient capital to seek early retirement, where he could expand his auto-education and pursue his own research and writing. This research has resulted in posting 160 linked papers on the Academia web site that are freely available to all.

REVIEWER'S WEBSITE

All of the reviewer's prior essays and other reviews (referenced herein) may be found, **freely** available at:

https://jamescook.academia.edu/HerbSpencer

1 INTRODUCTION

This essay draws heavily on the magnificent book: *Discovering Enzymes by* David Dressler (1991) in the Scientific American Library series. This summary is written to share crucial information about the cell's principal component: the **enzymes** that are the true **secret of life**. I have a skill, developed over a lifetime, of reducing vast amounts of complex information by a factor of at least 20, so that others may learn information without having to commit weeks of their time to reading the sources I have digested; for example, *Enzymes* is a book of over 260 pages that I cherish. It has proved invaluable in extending my own knowledge of living systems.

1.1 CENTRAL ROLE IN LIFE

Dressler is an excellent teacher and sets the scene for the key role of enzymes through their key role in every living cell by introducing them as powerful molecular machines that can construct complexity from simple atoms. Astonishingly, this (unfinished) story has evolved only over eighty years.

He sets the scene with a retelling of the present theory of material evolution: from stars, to planets, to rocks with an emphasis on the fortunate circumstances of the Earth as a suitable sized planet, moving around the sun in a stable orbit at a suitable distance to permit water to exist in all three forms: liquid, gas and solid. The radioactive nature of the earth's core led to frequent volcanic eruptions of gases that were washed by rain into the ocean. Life always seems to need liquid water to promote appropriate chemical reactions. Without going into the fundamental mystery, Dressler goes (in a single sentence) from aggregates of matter growing by random accretion, to a more orderly and exact process of reproduction to produce the first living cells. He emphasizes that the molecular components of living protoplasm are of much greater size and much more organized than the molecules produced by chemical transformations alone [see my *CHAINS* essay]. The key to this final step is the invention of a remarkable set of microscopic machines called **enzymes** that are specially shaped macro-molecules that are twisted into the needed shapes to select out and transform only certain kinds of other molecules. Here, the electrons moving around certain atoms can pick out orbitals that generate complex interactions that in their thousands, work together, to produce **life** by absorbing simpler molecules from their environment, getting changed into a range of dynamic structures from bacteria through plants to animals. Each example can persist for an extended period (a lifetime !) until they breakdown and recycle their atoms back into the larger game. The magnificent trick was to remember with certain large molecules (**DNA**) that could participate in preserving the various stable structures that we have called species [see *CELL-SPECIES* essay]. The miracle is that inert chemicals (a few types of atoms) have been able to create islands of life within a vast chemical universe. Too many scientists use Magic words, like Evolution to imply they understand these processes: **they do not**.

1.1.1 CELLULAR GROWTH

When a single bacterium cell is cultivated in a laboratory and provided with 'food': a suitable medium of sugars and a few simple salts (sodium sulfate and ammonium chloride), the cell cannot just survive but thrive by dividing roughly every hour. This process will continue as long as sufficient 'food' is provided by the transformation of a few simple molecules into living protoplasm. The secret is the cell has its own set of chemical transformers, called **ENZYMES**, that are responsible for carrying out **all** of the chemical reactions of the living cell across multiple generations; they are the Life-Force of the cell.

Proteins play two mutually complementary roles within cells: they are both the raw material **AND** the processing engines (called Enzymes) that manipulate the processes involving proteins. Even bacteria contain from 50,000 to 2 million proteins. Truly nucleated cells (**eukaryotic**), like us, are larger and contain much more protein: one yeast cell is estimated to contain about 50 million proteins, while human cells carry from one to three billion molecules of protein [NB biochemistry always involves huge numbers]. Some of these proteins are short-lived and unique at any time, whereas others are so basic that millions of examples (copies) are needed. The actual proteins in a given cell depend on its type (over 200 hundred in humans - see my *Cell* essay].

Enzymes work by transforming (altering and re-arranging) the simple molecules of the environment, one step **at a time** [but thousands of parallel processes **simultaneously**] to form the many complex molecules that are **ALL** needed for the cell to survive. For example, while many of the sugar molecules taken from the growth medium are completely dismantled (to release chemical energy), others are only partially torn apart and the pieces recombined to generate a family of related small molecules. When these new molecules are further transformed (by other enzymes) by the addition of nitrogen or sulfur atoms, taken from the salts, then the cell has created about 100 small organic (based on carbon) molecules that serve as the universal building blocks - the amino acids, the sugars, lipids (fats) and finally the nucleotides (DNA). These building blocks are then used in a second cycle of biosynthesis to construct all the larger, more complex macromolecules of living protoplasm: all controlled by even more complex enzymes. These larger macromolecules have the potential for exhibiting special, useful physiological properties. For instance, one basic group (the lipids) help form membranes or a boundary around the whole cell as well as smaller parts, called organelles [see *Cell* essay]. Thus, the role of the enzymes in the life of every cell is to mediate the transformation of the simple chemical world of the raw environment into living protoplasm. They transform simple molecules across the threshold of life [Sorry, folks we are all just chemical factories !!].

Higher organisms are similar to a single cell bacteria with important differences. Here, the individual cells are aggregated together (to assist in co-operative activities) so each one is not in direct physical contact with its external environment. This means that each cell must be supplied with special solutions created for all of them, by the organism as a whole. This requires **digestion** that needs a major fraction of the system to produce a food processing tube (the digestive tract) with multiple sub-systems like the liver, the stomach and the pancreas. These internal organs perform specialized actions to break down the raw inputs (plants or other animals). So, for example, near the tubes entrance, salivary glands supply enzymes breaking down the carbohydrates of grains and fruits into free sugars; farther along, certain stomach cells secrete enzymes that break down meat protein into their building blocks (amino acids) - [see *Proteins* essay]. These are then absorbed by cells lining the digestive tube and passed into the blood (circulatory) system. From the bloodstream they are taken up into the individual cells and reassembled into the specific macromolecules that are needed in each organ. These form the central processes of **Metabolism**.

1.2 CELL SPECIALIZATION

In contrast to the unlimited urge to grow, exhibited by bacteria, multi-cellular animal cells in a rich food environment generally stop dividing within a short time (5 to 50 generations). Uncontrolled growth in a higher organism is ultimately lethal, as demonstrated by the uncontrolled growth of cancer tumors. This is because the component cells of higher organisms are members of a larger, cellular community, with each type of cell having its own assigned responsibility and function, so this limits the growth of any specialized cell type, as it is the specialization and diversity of the cells in higher organisms that is their critical function. The key to cell specialization is Enzyme-Specialization. In addition to the basic set of enzymes needed to maintain the life of the cell, each cell type also makes its own unique set of enzymes, to achieve the specific goals of that cell. Some of these specialized enzymes first construct the diverse structures of the cells while others then direct these cells to do their own special tasks as well as metabolism to stay alive. For example, looking at nerve cells (or neurons) that play a key role in integrating all the functions of higher organisms, ranging from sensory perception, to decision making, to muscle movement (both involuntary - as in the heart or actively in moving our fingers). Most of a neuron's mass is concentrated in its cell body, where the metabolism is concentrated to keep all of the cell alive. This is attached to a long fiber (or axon) that carries the signal from one cell to many others. The technique is centered in the axon's membrane, where specialized enzymes generate a difference in the electrical charge between the outside and inside of the cell by pumping two types of ions across the membrane. In the resting state, an excess of positive sodium ions exist in the fluid surrounding the cell. When a nearby cell is stimulated the permeability of the membrane is altered and sodium ions enter quickly that collapses the charge differential; this change is moved rapidly down to the end of the axon.

Every neuron is **not** physically in contact; it ends in a vesicle filled with a small signaling molecule (called a neurotransmitter) that drifts across the gap (the synapse) to the next cell, where there are 'capture' molecules (receptors) that are triggered by the arrival of a neurotransmitter molecule; this triggers further local action. After completion, all these parts must return to the 'ready' state that is achieved by more enzymes. It is important to realize how this process may be disrupted by toxic substances that interfere with the several enzymes involved. For example, the neurotoxins of the cobra snake and the puffer fish block two major ion channels, while many deadly nerves gases inhibit the enzyme that controls one of the body's major neurotransmitters, like acetylcholinesterase.

1.3 PHYSIOLOGY

Not only are specialized organs defined by specialized enzymes but so too are the major physiological **processes** that characterize the organism as a whole. A good example is blood clotting. With the evolution of large multicellular organisms, it was necessary to develop a system wide circulatory system [see *Organs* essay]. This introduced a major risk of a break in the piping system, where the animal could then bleed to death in minutes. The defense against this mechanical disaster was a set of inter-connected enzymatic reactions leading to the temporary formation of a blood clot. In brief, a set of enzymes change the character of blood from liquid to a solid in any region, where a leak has occurred.

The key event involves an enzyme called fibrinogen that is usually inert but when a leak occurs then 13 distinct enzymes work in sequence to convert fibrinogen to **fibrin** that are then compelled to join their long fibers together to form a sticky mesh that traps large numbers of blood cells to form a strong, gelatinous blood clot to block the leak. As each enzyme in the series activates hundreds of copies of the next enzyme, an ever-expanding 'cascade' culminating in a blood clot being made in seconds. Research has shown that a genetic malfunction results in the disease of **hemophilia** where blood fails to clot properly because a single gene fails to make one of the 13 needed fibrin-forming enzymes.

1.4 DEVELOPMENT

There are several times (or phases) in the life cycle of an organism when enzymes play different roles. One of the most important is in the Growth Phase (or Development) during which needed changes are triggered and managed. Dressler briefly describes part of the life cycle of a moth going from caterpillar through cocoon. The cocoon is a protective, self-made environment wherein the caterpillar spins a macromolecule of silk (a typical cocoon needs about one mile of silk to be synthesized). Once complete, the caterpillar completely reorganizes the tissues of its body while undergoing dramatic developmental changes that transform it into an adult moth. When ready, a specific enzyme is produced that digests the cocoon and frees the moth. This is accomplished by the salivary glands of the maturing moth that produce *cocoonase*, an enzyme whose sole purpose is to digest a hole in the cocoon that grows until the moth can escape. This story of the moth illustrates that at each stage in the development of an organism the actions are guided by specific enzymes. Dressler makes the claim that all the examples of vital enzymes illustrated in his prologue (summarized here) are members of one family of enzymes, which has evolved from a single ancestral enzyme. This enzyme, *chymotrypsin* becomes the star of this story and is summarized here.

2 DISCOVERY OF ENZYMES

2.1 HISTORY

Aristotle heavily influenced earlier thinking, with simple analogies. He proposed that inanimate objects were similar to living creatures that had a natural goal toward which evolved over time: this was his simple metaphysical theory of Time and Change [see my essay on *Change*]; the acorn's goal was to become an oak tree. So, immature grape juice was destined to become wine and the over-ripening of wine was vinegar (or death). All of this 'change' was due to the "*Vital Force*". This was the mysterious Fifth element ('*Quintessence*'; in addition to Earth, Air, Fire and Water) whose variants explained the wide variety of 'stuff'. The educated men (with their good memories, dedicated to remembering their lessons of history) venerated Aristotle, whose ideas were unchallenged for 2,000 years [a good lesson for today's arrogant scientists].

Many quintessences were thought to be gaseous that could be expelled by heating and later recovered by condensation. When this was used with wine juice, a clear substance was extracted, they named *'aqua ardens*' (or 'burning water', that we now call alcohol, actually ethyl alcohol or **ethanol**).

2.2 FERMENTATION

The discovery and early history of enzymes was intimately bound up with the process of alcoholic fermentation - making wine and beer. The move to modern science was the introduction of mathematics (really, only arithmetic) by Antoine Lavoisier (1734-1794). As a French accountant and government tax-collector, he was inspired to guess that when substances combine or break apart, they do so in specific amounts (or ratios). He also guessed that the total amount of mass (like money) was preserved overall. He concluded (contra Aristotle) that after a reaction, a new substance had been created and not just the properties had been altered (or 'matured'). At the same time, an English self-taught school teacher, John Dalton (1766-1844) proposed an Atomic Theory of Matter that showed how compounds could be assembled from integral numbers of a few Elements, like oxygen, hydrogen, nitrogen and a few metals.

Lavoisier returned his attention to wine where it had been recently discovered that sugar accounted for 25% of the unfermented wine juice, so he correctly proposed that ethanol was the product of converting sugar but this required adding a small amount of yeast (or '**ferment**' as it was called) since grapes always had some natural yeast on their surface. This work was refined by another French chemist, Joseph Louis Gay-Lussac (1778-1850). In modern notation, he showed that:

$$C_6H_{12}O_6 \longrightarrow 2\{C_2H_5OH + CO_2\} \quad \text{or} \quad [6C+12H+6O] = 2[(2C+6H+O) + (C+2O)].$$

However, even though they solved the book-keeping problem, they had no idea Why? it was happening.

2.2.1 YEAST

The focus soon centered on the role of yeast. In 1839, the German chemist Justus von Liebig (1803-1873) proposed that yeast was a highly dynamic (vibrating) substance that would break up the sugar molecules, releasing the carbon dioxide gas (CO_2). At the same time, a young German physiologist, Theodor Schwann (1810-1882) claimed that the yeast deposited in the fermentation vats was, as seen with microscopes, was composed of living material. However, the claim that yeast was alive was rejected by the dominant materialist chemists of the time, as an unwarranted intrusion of the vague (non-mathematical) science of biology and relied on the poor optics of microscopes; indeed, a weak return to earlier 'vitalist' theorizing. It took almost 25 years before Schwann's correct theory was accepted that required Pasteur's research.

2.3 PASTEUR

Louis Pasteur (1822-1895) is viewed as one of the great scientists of the nineteenth century. He started out studying the optical properties of sugar crystals in transmitting light. He was invited to investigate several failures in making wine on an industrial scale. He first uncovered lactic acid, instead of ethanol. When he examined the yeast with a microscope he discovered that the vats that produced alcohol contained large globules of yeast, some even showing buds (growth) but there were no yeast globules in the vats with lactic acid. Pasteur guessed that the production of ethanol resulted from one microscopic organism (microbe) and the production of lactic acid resulted from another (different) microbe. He was able to cultivate both types separately; his suggestion of thorough cleaning of vats before each cycle was accepted and solved the problem. This led him to a more subtle view of wine fermentation with the discovery of tiny quantities of other bi-products, such as glycerol, butyric acid and succinic acid. He saw that yeast were collections of living cells. From all of these findings, Pasteur realized that living cells possess the ability to transform (or metabolize) numerous organic compounds to create a variety of other products. In other words, the growth of a cell is dependent on such chemical reactions; alternatively, such reactions were an expression of the life of the cell.

2.3.1 OTHER FERMENTS

During this time, several other chemical transformations were being discovered. In 1833, it was found that the water solution surrounding germinated barley grains (malt) contained a material that could decompose the starchy interior of seeds into free sugar. This new extract was called '*diastase*' (Greek for 'breaking').

In its purified form it functioned without the presence of any cells (like yeast). Schwann was investigating stomach digestion and found that gastric juices were capable of bringing about "artificial digestion" in a test tube. He was able to purify this and called it '*pepsin*'. Since these new transformative agents did not require the presence of intact cells, they were called 'soluble ferments'. It required an independent Polish chemist, Moritz Traube (1826-1894) to propose a unified theory that there existed in many cells, substances analogous to the known soluble ferments that were carrying out many undiscovered transformations. In effect, he proposed that chemical transformations in all life forms - not just micro-organisms - might be traced back to the actions of specific, reaction promoting substances that would function like a set of cellular 'machine tools'. In 1860, Traube's hypothesis received strong support from a French chemist, Marcelin Berthelot (1827-1907) who isolated a 'soluble' ferment he called '*invertase*' that could break apart one form of sugar (sucrose) into its two simpler sugars, glucose and fructose. He showed that invertase was special, in that it was a ferment extracted from the living yeast cell itself. It was a simple step to then suggest that a cell was simply a collection of ferments, each of which would ultimately be extracted and purified, like any other chemical substance.

2.3.2 PASTEUR'S MISTAKE

It was a German biochemist, Wilhelm Kühne (1837-1900), who supported Pasteur in this 'Science War'; he gave the group of soluble ferments their own name: *Enzymes*. This was invented to segregate the soluble ferments into their own small area of biochemistry. Eventually, this idea would expand to cover **all** chemical transformations in the biological world. Pasteur ignored the key insight: cells contain substances that promote various reactions and these substances can either function inside the cell **OR** be released and function in a suitable external environment. Like many other highly motivated scientists, he was probably reluctant to accept a theory that was not his own and was much at variance with his own way of thinking. Making matters worse, he would have to accept a theory that was associated with the chemical tradition of Germany, a country that had only recently (1870) initiated and won an aggressive war against France.

2.3.3 BERZELIUS and CATALYSIS

It was the Swedish genius, Jöns Berzelius (1779-1848) who discovered the correct formulation in his paper of 1837 that was largely overlooked. He discovered three of the elements (cerium, selenium and thorium) as well as inventing the modern system of writing chemical formulae, using the first letter of the element's Greek or Latin name and adding a subscript giving the number of that element in the compound (e.g. H_2O). With the invention of the electric battery in 1800, investigations of electricity flourished. He realized that electrical attraction and repulsion explained chemical attraction and repulsion, so that chemical reactions could be explained by their electrical re-arrangements. He observed that acids could influence a chemical reaction without being changed itself. Even the presence of an inert substance, like gold, could promote a chemical reaction, as in the peroxide (H_2O_2) reaction:

$$2\ H_2O_2 \longrightarrow 2\ H_2O + O_2$$

In consequence, Berzelius named these critical reagents 'Catalysts' that can accelerate the rate of chemical reactions. This tremendous contribution was ignored for 40 years as too many scientists were cantankerous and jealous of the success of their rivals.

2.4 BUCHNER and ZYMASE

The next advance began when Eduard Buchner (1860-1917), a Munich chemist visited his older brother Hans, a Munich bacteriologist, who was studying yeast. They noticed that after squeezing yeast at great pressure the resulting substance (they called *zymase*) was able to release carbon dioxide from sugar without a single yeast cell still present. Initially, this was a controversial result as few competitors could replicate this effect. This enabled enzymes to be studied in a test tube, rapidly enhancing the new science called Biochemistry. In 1907, Eduard Buchner was awarded the Nobel Prize in Chemistry; his brother did not share in the prize, as he had died in 1902 (and only living scientists win the awards). Thus, after half a century, Traube's original proposal had emerged as a central dogma of the new science of biochemistry: the whole metabolism of every living cell was defined by distinct enzymes.

Soon, it was realized that the conversion of glucose into ethanol was not done in a single step by a single enzyme, as Buchner had believed, but in numerous small steps that had to occur in a precise order with each requiring its own proper enzyme. So, it was seen that nature uses complex **processes**: a connected chain of reactions (or *metabolic pathway*), changing intermediate compounds of one atom or group of atom, each requiring its own enzyme. Now science could see the path forwards but the price was an explosion in overwhelming **complexity**. So, not only had the scientists got to expose the role of each enzyme in bringing about chemical transformations but how all these processes contributed to the physiological activities as digestion, blood clotting and information transmission in the nervous system.

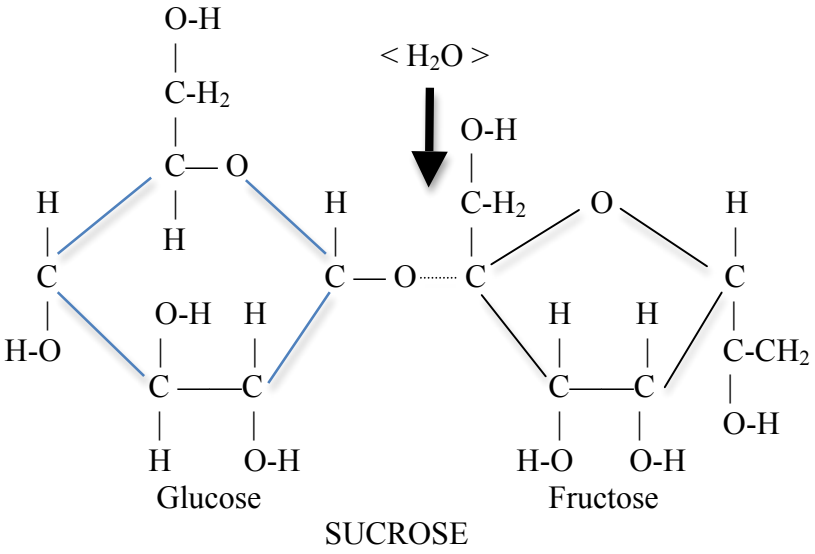
3 INVESTIGATING ENZYMES

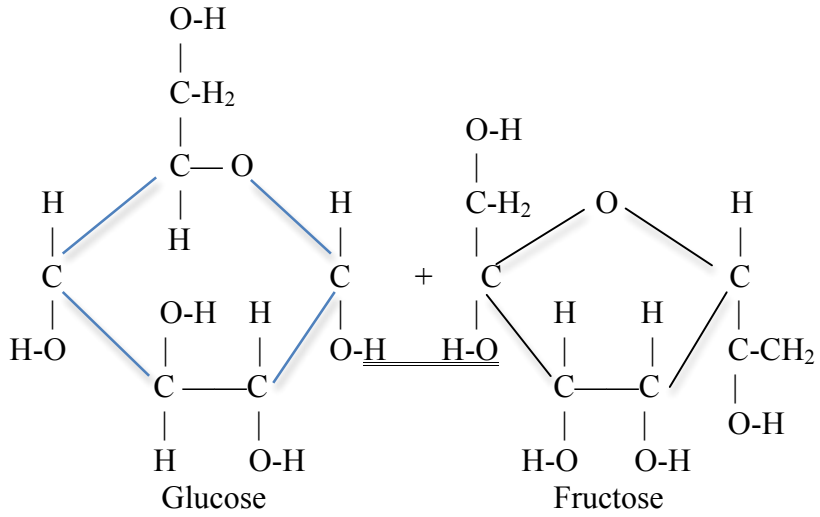
Although enzymes had been named, their nature remained a mystery. This required chemistry itself to be split into two complementary parts: inorganic and organic (or biochemistry). Within the original studies of chemistry, the elements were discovered and simple molecules could be explored through the mechanical means of temperature and pressure. However, in the world of living matter, molecules were much more varied and complex (and vastly larger); their reactions are controlled by enzymes that are readily destroyed by excessive heat.

3.1 NON-ENZYMATIC SUCROSE REACTION

Most biologic molecules are very stable, so that if we try to breakup sucrose into its two parts (glucose and fructose) by dissolving it in water then the reaction proceeds very slowly. If we use modern notation, to see the molecular structures, we see that sucrose is a bi-sugar, linking glucose and fructose through a single link of one oxygen atom [see below].

This reaction consists of *hydrolysis* (Greek: breaking by water) . The need is to split a water molecule across the 'joining oxygen/carbon bond, marked below by the dotted line). This results in the following split pair of molecules with the H_2O divided (as underlined).





That is: $C_{12}H_{22}O_{11} + H_2O \longrightarrow C_6H_{12}O_6 + C_6H_{12}O_6$

3.2 ENZYMATIC SUCROSE REACTION

Without an enzyme or acid/alkali, the sucrose dissolves in water incredibly **slowly**: even years at room temperature but somewhat faster using hot water. Like most molecules found in nature, they are very stable; hard to make and hard to break. Nature accelerates this with a single enzyme, Berthelot's invertase (or *sucrase*, using modern naming conventions). It is found in yeast that is a fungi that live off decaying plant material. In animals, sucrase is attached to the outer membrane surface of the cells lining the small intestine, where it is used to break down sugars that are absorbed directly into the blood stream. A tiny amount will accelerate the reaction by a factor of billions, completing the reaction in minutes, not years, while heat alone will only speed up the reaction by about one hundred fold. Heat soon destroys the enzyme; maximizing its effect at about 50°C but switching it off above 60°C, when the enzyme is destroyed.

3.3 AMINO ACIDS

It was Liebig who made the first important step who found that acid could break up *casein* (the solid albuminoid derived from milk) into *leucine* ($C_6H_{13}NO_2$) and *tyrosine* ($C_9H_{11}NO_3$). Eventually their complete chemical structures were obtained. They shared a similar base structure [NH_2CHCO_2H] plus a variable addition (later called a Side-Chain): Leucine's was [$CH_2CH(CH_3)^2$] and tyrosine's being [$CH_2C_2(C_2H_2)^2OH$]. The base structure [$NH_2CHCOOH$] was a central carbon atom (C) which are attached a hydrogen atom, an amino group [NH_2] and a carboxylic acid group [COOH]. Later, leucine and tyrosine were found to be released from many proteins upon acid treatment and their presence soon became a diagnostic test for protein. So, leucine and tyrosine became the forerunners of a set of 20 similar compounds that came to be known as **amino acids** as they all had the same base structure but different side-chains. The present list of amino acids was not completed until 1935 but only 20 were found in living cells [see *Protein* essay].

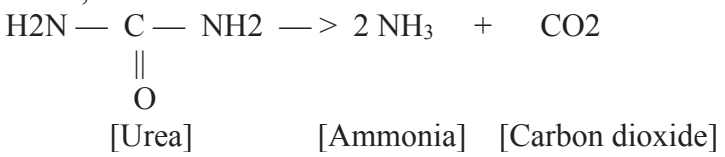
3.4 POLYPEPTIDES

The understanding of proteins as polypeptides did not occur until 1902 with the work of Franz Hofmeister and Emil Fischer. They proposed that the intact protein is a chain of amino acids joined together by strong bonds between the regularly repeating amino and carboxyl groups [see *Chain* essay], shown here { =*= }. The resulting chain (or polymer) of linked amino acids was termed polypeptide chain, such as:

Lysine =*= Leucine =*= Leucine =*= Tyrosine =*= Aspartic acid.

The central role of proteins as enzymes in living organisms was not fully appreciated until 1926, when James Sumner (1887-1955) showed that the enzyme urease was a protein. Urea is the major end product of nitrogen excretion in mammals (converting ammonia from the breakdown of amino acids).

Urease is the enzyme that assists in decomposing one molecule of urea into two molecules of ammonia and one molecule of carbon dioxide, as in:



This decomposition reaction allows plants to unlock the nitrogen present in animal waste for later use in its own metabolism. Sumner extracted urease from plant mulch then concentrated them using salts and very cold temperatures to produce crystals that could be melted to activate the above reaction. Sumner's results only gained full acceptance about ten years later when two researchers (John Northrup and Moses Kunitz) at the Rockefeller Institute obtained crystals of a few digestive enzymes (chymotrypsin, trypsin and pepsin) using similar freezing techniques. Both Northrup and Sumner shared the Nobel Prize for chemistry in 1946. This work showed that the remarkable catalytic activity of enzymes was hidden in the amino acid sub-units of the giant polypeptides called proteins.

3.5 CONSTRUCTING PROTEIN POLYMERS

Polymers are one of nature's favorite constructions; they are **chains** of units (called monomers) that join together at specific points: amino acids join to form proteins just by making universal **peptide** bonds; often this is done in a Condensation Reaction, in which a molecule of water is lost with each subunit added. In the case of building proteins, it is vital that the right sequence of monomers are added. This is why cellular enzymes are more intelligent than factory machines that must rely on a human to control the sequence. The complementary reaction, 'breaking' (or cleaving a covalent bond by adding water) is called Hydrolysis.

3.5.1 SUBSTRATES

A crucial step in enzymatic reactions is overcoming an energy barrier by binding to an input molecule, called a **substrate**, holding them in place that requires less energy to complete the process (or activation). The result of a successful enzymatic transformation is called the **product**. The enzyme usually remains unchanged after helping the reaction, ready for the next substrate molecule.

Enzymes are very selective in the substrates they work on by having active sites (or grooves) where the substrate fits ("lock and key"). Catalysts increase the rate of chemical reactions because they allow a much greater completion rate than if only random collisions occur; sometimes by a hundred trillion times (10^{14}). An enzyme can often catalyze the reaction of thousands of substrate molecules every second (its 'turnover rate') so it must be able to bind a new substrate molecule in a fraction of a millisecond.

4 STRUCTURE of CHYMOTRYPSIN

Proteins begin as linear chains (called the Primary form) as amino acids are added serially or from two linear proteins. It is defined by its list of component amino acids, beginning with the nitrogen end. Proteins cannot provide high-level functions (like enzymes) in this too simple form but this is how they begin. Often, other enzymes speed up the buildup the proteins from simpler amino acids.

4.1 LINEAR 3D SHAPES

It was soon realized that real enzymes are a lot more than a Giant Protein; their **shape** is critical to their activity: what substrate they attach to and then how they interact. Dressler selects out one particular enzyme, *chymotrypsin* to illustrate many of these perspectives. He selected chymotrypsin because it has been under continuous study for over one hundred years, so that more is known about its structure and mode of catalysis than for any other enzyme. It is readily available as it is produced in large quantities in the vertebrate digestive system by the tiny *acinar* cells in the pancreas. It is released into the pancreatic duct in an inactive form but then activated when it reaches the small intestine, where it breaks large proteins into moderate sized pieces that are then further degraded into amino acids by other protein-digesting enzymes (called proteases). These digestive steps are done in minutes with enzymes but would take many years without them.

Like most proteins, chymotrypsin contains all 20 amino acids. There is nothing unusual about this composition of 241 amino acids: they vary from a few (methionine = 2, histidine = 2, arginine = 3, tyrosine = 4, glutamic acid = 5, phenylalanine = 6); to quite a few (tryptophan= 8, proline = 9, aspartic acid = 9, cysteine = 10, glutamine = 10, isoleucine = 10) to many (asparagine = 13, lysine = 14, leucine = 19, alanine = 22, threonine = 22, glycine = 23, valine = 23, serine = 27). As I discussed before [see *Proteins* essay], the nature of the side-chains are critical in determining how all these amino acids fit together. Six of them (glycine, alanine, valine, leucine, isoleucine and proline) just have simple hydrocarbon side chains. These structures are *hydrophobic* ('water-fearing') so they prefer to fit together, with their side chains interacting with each other rather than remain exposed to the surrounding water molecules. The larger the hydrocarbon side chain (e.g. Isoleucine) the more pronounced is their hydrophobia. Two more amino acids, serine and threonine, also have short hydrocarbon side chains but these end with a hydroxyl group (-OH) that can participate in the formation of hydrogen bonds. There are three amino acids whose side chains are planar hydrocarbon rings (phenylalanine, tyrosine and tryptophan). They are quite hydrophobic but the planar atomic arrangements encourage unusual geometric arrangements different from the simpler, branched side chains. In addition, tyrosine and tryptophan side chains contain OH and NH groups that can form hydrogen bonds. The next five amino acids (lysine, arginine, histidine, aspartic acid and glutamic acid) are more acidic that makes them more *hydrophilic* ('water-seeking') and these also can form ionic bonds. There are two variants, asparagine and glutamine, in which a terminal amino acid group (NH_2) has replaced the singular oxygen atom in the branch side chain's carboxylic group that prevents them forming ionic bonds. There are two amino acids (methionine and cysteine) that contain sulfur in their side chains. Methionine's sulfur is tucked away behind a methyl group so is not very active but cysteine's sulfhydryl group (**-SH**) is very active and is attracted to a matching one in another cysteine amino acid to form a very strong (covalent) *disulfide bond*.

However, the strongest links in the protein are the peptide bonds linking the primary amino acids together always leaving one amino group at the end of a chain (taken to be the start - by convention) and one free carboxyl group (taken to be the end of a polypeptide chain). In the case of chymotrypsin, the 241 amino acids of the completed enzyme are distributed into three distinct polypeptide chains: A=13 units, B=131 units and C=97 units long. When chymotrypsin is first generated in the pancreas, it is first assembled as a single polypeptide chain but once it reaches the small intestine it is cleaved in two places to produce three sub-chains that are joined together by two disulfide bonds that can be chemically unfolded by using urea.

4.2 SEQUENCING PROTEINS

The major contribution to determining the amino acid sequence were developed by Frederick Sangster, who was awarded his first Nobel Prize in 1958 by first selecting the unattached initial amino ammonia (NH_2); this destabilizes the next peptide link releasing the first amino acid. This can be repeated over and over, to determine up to 40 amino acids in the shorter chains.

4.3 ENZYME 3D SHAPES

The enzymatically active form of a protein requires the linear sequence of the polypeptide to mutually self-interact to **fold** into a unique 3D shape ('Conformation') whose shape and aligned amino acids will select only a few matching molecules (the target substrate). The first change produces a secondary shape when nearby areas of the backbone of the polypeptide chain create local regions of folding. In the second, it needs interactions between specific amino acid side chains bring distant areas of the polypeptide chain together.

4.3.1 ALPHA HELIX

The simplest 3D form of many proteins (at least parts of them) are helical, called **alpha-helix** (α helix); it was found in keratin (abundant in skin, hair, nails); [see *Proteins*]. The first insights were obtained in the 1940s by Linus Pauling at CalTech using X-ray diffraction on short protein chains of crystallized amino acids. Some proteins, like myosin, fibrinogen and keratin are useful in this form.

The alpha-helix is constructed from a hydrogen bond (in the N-H) to the C=O in four peptide bonds away in the same chain forming one complete turn every 3.6 amino acids. In some proteins, α helices wrap around each other to form a coiled-coil (a very stable structure). This shape forms when two or three a helices have most of their nonpolar side-chains on one side, so that they can twist around each other with each other with these side chains facing inwards. Long rod-like coiled-coils provide the structural framework for many elongated proteins as in skin and muscles.

4.3.2 BETA SHEET

The other standard pattern, also proposed by Pauling, is called a **beta** (β) sheet that appears like long, parallel ribbons, sometimes in the same direction (parallel) or in alternate directions (anti-parallel). It was first found as fibroin in silk. Several β-ribbons can align in a single sheet. As both of these forms arise from hydrogen bonding across peptide bonds in the polypeptide backbone, so that many different amino acid sequences can form them.

4.3.3 DISULFIDE LINKS

The alpha and beta structures are mostly used in structural proteins but for enzymatic proteins a different method is used to link two **sulfur** atoms in distant cysteine amino acids into a very stable disulfide bond. There are millions of interactions possible among the amino acids of a long polypeptide chain; for example, only considering the 10 cysteine units in chymotrypsin, there are more than 1000 different ways to form just disulfide linkages. When we add in the other possible linkages between side chains it becomes impossible to predict the final 3D shape, the following image has been obtained of 4,000 atoms in a 'space-filling' model. Further cross-sections show that the 3D structure is highly asymmetric. Dressler shows more details. Here is a (computer-generated) space-filling model of **chymotrypsin**; complicated? YES !!

4.3.4 BINDING SITES

The folding of the polypeptide chain often creates a **crevice** or cavity on the protein surface (its binding site). This crevice exposes a set of amino acid side chains arranged in a specific manner that they can form weak chemical bonds only with certain ligands whose atoms match the shape. This optimum shape is enhanced if a number of negatively charged side chains come close together then the affinity of the site for positively charged is greatly increased. Also, when several nearby parts of the polypeptide chain restrict water molecules from a region then the site becomes more attractive to a suitable ligand as water molecules readily form hydrogen bonds that can compete with ligands for sites on the protein surface. Conversely, at a later stage, allowing water into these sites can weaken the hold on the ligand and finally release it from the protein when the interaction is finished, releasing a modified substrate molecule for the next step.

5 ACTIVITY of CHYMOTRYPSIN

The reason Chymotrypsin is seen as a master enzyme is that while its set of amino-acids remains fixed, its overall shape may change by adjusting the interactions between adjacent surfaces of the protein to highlight the selection of different substrates. This flexible strategy allows chymotrypsin to range far afield from its original role in digestion, as it participates in several complex physiological processes, like blood clotting and neuronal activity; as we will describe (in detail) next.

5.1 TRYPSIN

Judged from its reaction mechanism alone, this primary catalytic active site should be able to cleave any peptide bond in any protein (far too powerful); yet chymotrypsin, like all enzymes, is remarkably specific with respect to the compounds it works on. Along with its tremendous **catalytic** power, this **specificity** is the second hallmark of enzyme action. The key to the enzyme's specificity lies in an area of the protein adjacent to the catalytic active site; an area called the substrate-recognition-site. This is the part of the enzyme that is responsible for the **'lock-and-key'** fit with the substrate. In chymotrypsin, it ensures that only peptide bonds adjacent to phenylalanine, tyrosine or tryptophan will be cleaved. This analysis of the substrate recognition site has shown that the enzyme and its substrate are complementary in shape, proving Fisher's original concept.

The enzyme ***trypsin***, like chymotrypsin, is a digestive protein produced by the pancreas. It differs from chymotrypsin with respect to the amino acids it attacks in a target polypeptide chain, cleaving after positively charged amino acids, such as lysine and arginine, rather after the large, planar amino acids phenylalanine, tyrosine or tryptophan. Trypsin complements chymotrypsin in the degradation of proteins in the small intestine: when ingested proteins are acted upon by both chymotrypsin and trypsin, they are broken into smaller fragments than would be produced by either enzyme alone; this speeds up digestion.

A detailed comparison of these two enzymes shows how they are related with very similar sequences of amino acids. Trypsin also has about 250 amino acids and if laid out next to one another, an almost perfect alignment can be achieved; at each position there is about a 50% chance that the amino acids are the same. This degree of homology is even more impressive, for when the 3D structures of the two are examined, they are found to be almost identical. Most of the differences lie on the surface of the 3D structures - where the projecting amino acid side chains interact.

5.1.1 SERINE PROTEASES

In both enzymes, the key catalytic amino acid, **serine** is located at approximately position 195 in the linear sequence. Collectively, these enzymes are referred to as serine proteases, denoting the key role of this part. The subtle differences are sufficient to allow the enzyme to react with a different substrate while continuing to use the same catalytic mechanism. Assuming that chymotrypsin was the primordial protease, a single mutation could have changed Serine -189 to aspartic acid to create the enzyme trypsin. Indeed, the modern consensus is that the modern forms of both chymotrypsin and trypsin have evolved from a common ancestral protein.

5.1.2 ELASTASE

Whereas chymotrypsin and trypsin cleave after target amino acids in any protein, there is another similar enzyme ***elastase*** that has evolved a higher degree of specificity that only targets the protein *elastin*. This is an important protein in a number of body tissues, serving as a major structural element in ligaments and in the walls of blood vessels. The basic **elastin** molecule is a 700-amino acid polypeptide with a high content of hydrophobic amino acids (like alanine, glycine, proline and valine). The side-chains are enzymatically joined together by unusual lysine-lysine cross-links, so that thousands of individual elastin chains become parts of a larger covalently connected structure, forming a mesh-net. The enzyme system that cross-links elastin fibers continues to increase as we age; the resulting reduction in elasticity of arterial walls may be a contributory factor to cardiovascular disease [perhaps, part of nature's planned obsolescence?].

Elastin is made both by the pancreas for digestive functions and by white blood cells for tissue remodeling. This enzyme is another variation on the evolutionary theme of chymotrypsin and trypsin as its 250 amino acids align with each of them. It only differs in its substrate recognition site, which has been modified to accommodate the small amino acids glycine and alanine as targets; specifically, Glycine-216 has changed to valine and Alanine-226 has become threonine.

5.1.3 PRO-ENZYMES

Proteolytic enzymes are very powerful agents and must be carefully controlled within the body; this has produced two major methods: Pro-enzymes that involve the synthesis and storage in an **inactive** form and also the synthesis of anti-proteases that are non-enzymatic **blocking** proteins that specifically interact with their respective proteases in a lock-and-key manner to deactivate them until needed. The serine proteases are too powerful to become active as soon as they are synthesized, so they are first made as inactive precursors (or pro-enzymes). Their activation occurs by the removal of a few specific amino acids from the linear protein form that blocks the final folding stage. Thus, when chymotrypsin is made in the pancreas, it is a single chain of 245 amino acids, called chymotrypsinogen. Activation occurs after it reaches the small intestine by an already active protease in the intestine that cleaves the pro-enzyme between Arginine-15 and Isoleucine-16 and then Serine-14 and Arginine-15 are trimmed back from the newly exposed carboxyl end. In a similar fashion, a second cut is made after Asparagine-148 and Threonine-147. This exposes the three sub-chains of chymotrypsin (namely, A, B and C) but the new shape allows additional structural changes until it reaches it final form. NB The suffix '**ogen**' is added to indicate the inactive (pro-enzyme) form.

5.1.4 ANTI-PROTEASES

The activation of a pro-enzyme is irreversible, so it can only be done once in the life-cycle of a protein. This is where the anti-protease comes in; when an enzyme can be switched off. In the case of serine proteases, this is done by the synthesis of specific inhibitory proteins, called Serpins (for SERine Protease INhibitors) that bind very tightly to the protease active sites to block their activity. A classic example is the serpin directed against elastase. It is produced in the liver and secreted into the blood, where it regulates the elastase released from white blood cells during the various repair and remodelling activities that follow injury and infection. This process protects tissues from digestion after the tissue has healed, so the elastase have no further function. Some individuals carry a heritable mutation in their anti-elastase gene that reduces the secretion of the inhibitor from their cells, reducing its quantity to only about 15% of normal concentration. As a consequence, an uninhibited overactive elastase destroys exposed elastin networks, particularly in the walls of the alveoli (the small air sacs in the lungs). Gradually, this condition develops in to the disease **emphysema** that seriously reduces the victim's breathing. This is made worse by cigarette smoking as smoke ingredients react with a methionine subunit that sticks out of the surface of the inhibitor.

5.2 BLOOD CLOTTING

Like other systems in the body, blood is composed of cells - red blood cells that carry oxygen and white blood cells that fight off infections. Unlike most organs, blood cells are not organized into a solid tissue: they float freely in their surrounding fluid, called **plasma**, which is a complex collection of substances, such as dissolved small metabolites, lipids, hormones, etc. Whereas damage to most organs can remain isolated to a small region, damage to the circulatory system is inherently systemic, affecting the entire body - so it is potentially life-threatening. This was briefly covered earlier [§1.3], here we will expand on the key function of fibrinogen and its enzymatic enhanced transformation to fibrin.

5.2.1 FIBRINOGEN

Before clotting occurs, fibrinogen is simply a large **inert** blood protein composed of six polypeptide chains (alpha, beta and gamma - interwrapped as two triple helixes) joined together at their amino-terminal ends by a network of (covalent) disulfide (S-S) bonds, forming a thick central core (or knot). After about 100 amino acids, these two helical areas terminate with the ends of the three chains forming closely aligned (but individually folded nodules) that create thickened terminal domains. The resulting fibrinogen protein is not spherical but more of an elongated cylinder: three nodules joined by two connector arms.

As a free protein, fibrinogen is soluble as it floats through the blood; the disulfide knot and the two terminal domains are negatively charged; so that they mutually repel each other. However, the properties of fibrinogen are changed dramatically if about 3 percent of the amino acids are removed from the knot. The removal of about 20 amino acids from the N-terminals of both the alpha and beta chains then releases material with a high content of negatively charged aspartic and glutamic acids; this changes the core knot area from -8 to +5 and its surface properties are altered significantly to attract the still negative (-4) terminal domains. These, called fibrin monomers, now interact with one another [see *Chains* essay] in a side-by-side and end-to-end fashion: a 'polymerization' set of events that produce increasingly long and sticky protein fibres. Fibrin monomers are held together initially only by weak non-covalent interactions (hydrogen and hydrophobic bonds). Gradually, the individual monomers become more cross-linked to one another through the action of a blood-clotting enzyme that covalently connects the side-chains of specific glutamine and lysine subunits. The resulting fibrin cables form a **mesh** in the localized region of the circulatory system, where fibrinogen has been converted into fibrin that traps large numbers of other blood cells to form a strong, gelatinous blood clot consisting of only 1% fibrin.

5.2.2 THROMBIN

The proteolytic cleavage that converts fibrinogen into fibrin monomers is triggered by an enzyme called thrombin that is always present in small amounts, in an inert form. Thrombin becomes activated (becoming a protease cleaving the four arginine-glycine sites) at the end of a dozen enzymatic changes in a complex sequence of events, beginning with the damage to the vascular system leading to aggregation of *platelets* at the injury site. **Platelets** are small oval cells (derived from huge bone marrow cells, called megakaryocytes) that adhere tightly to the protein fibres constituting much of the connective tissue between cells. Thrombin is a serine protease (a member of the chymotrypsin family of enzymes) with catalytic triad sites containing serine, histidine and aspartic acid plus a surface to stabilize the transition state. Thrombin is also very specific in cleaving only at arginine-glycine linkages that strongly resembles trypsin; this prevents the approach of any arginine not adjacent to glycine. Here, thrombin exhibits the evolution into a complex regulatory protease from a general digestive protease, such as chymotrypsin, trypsin, or elastase. The use of a serine protease to convert fibrinogen is used as an example of evolution using a basic enzyme design to solve a new physiological puzzle; [however, it is hard to see that stomach metabolic processes evolving prior to the need to have a workable (non-leaking) blood circulation system.]

5.2.3 VITAMIN K

The inactive form of thrombin (called pro-thrombin) is much larger than an ordinary serine protease; it is secreted into the blood from its source as liver cells. It appears as a polypeptide chain of 600 amino acids, of which only the final 250 are relevant to its serine-protease activity. The N-terminal half of the protein chain folds into several independent domains, each with a different function. One of these domains is used to bring the thrombin pro-enzyme to its site of action, where the unpredictable injury location has occurred. This is the function of the first 35 amino acids, with its ten negatively charged glutamic acid subunits which are carboxylated (the addition of a second carboxyl group [COO] to the ends of their side chains). This is carried out by an enzyme system that uses the small molecule, vitamin K as a helping factor that bind a calcium ion (Ca^{++}) that helps bind to phospholipid membrane proteins released by damaged platelets. This causes the whole protein chain to anchor at a specific site where blood platelets are responding to an injury. This tight binding brings pro-thrombin into close proximity with the blood-clotting enzymes that are responsible for its activation. The activation of thrombin occurs when the pro-enzyme (with its 600 amino acids) is cleaved at two sites. The pro-enzyme is first cleaved at the arginine-isoleucine bond between subunits 323 and 324. This generates active thrombin like with chymotrypsin. This cleavage allows the formation of an ionic bond similar to the one between the positively charged group of Isoleucine-16 and the negatively charged side chain of Aspartic-194. This new electrostatic interaction induces localized changes in the folded protein, forming the binding pocket and reorienting certain parts of the catalytic active site. Freed of its anchor, the activated thrombin floats away from the injury site and into the nearby blood, where it begins to trim fibrinogen molecules into fibrin that are capable of natural assembly into filaments.

5.2.4 ENZYME CASCADES

Rather than initiate thrombin directly, a long sequence of events leading from vascular injury to thrombin activation, involving the sequential activation of about 10 'blood-clotting factors' are triggered. All of these factors are members of the chymotrypsin family of serine proteases and, like thrombin, they are all produced in the **liver** and released into the bloodstream as inert pro-enzymes. In response to tissue damage and the aggregation of platelets at the injury site, the first blood-clotting factor becomes active. It is a serine-protease, it carries out a cleavage reaction on the second factor; which in turn, transmits the signal forward by further activating the pro-enzyme of the third component in the system until ultimately the proteolytic activation of the key blood-clotting enzyme, thrombin. The secret is that going from one level to the next, about 10 extra factors are activated, not just one. Thus, after the N^{th} factor is active, it has activated over 10^N others, so after 10 levels (N=10) there are over 10^{10} (about 10 billion) thrombin molecules activated. A huge army of responders to the dangerous leak in the blood circulation system. Sadly, a few people inherit a failure in the gene that produces factor VIII, so the chain is broken and they are at risk of bleeding to death: the horrible fate of hemophiliacs. Now factor VIII can be purified from normal blood and given to such people so that today most can lead a normal life.

5.2.5 PLASMIN

It is vital that the rapid and powerful cascade leading to the activation of thrombin not be allowed to get out of hand. The conversion of fibrinogen to fibrin must be confined to the injury site and not be permitted to extend into the surrounding circulation; this involves several strategies with the most important involving an anti-protease called anti-thrombin that always exists in the blood. Active thrombin molecules are being progressively diluted and inactivated. Only at the injury site is the number of active thrombin molecules high enough to overcome the reservoir of anti-thrombin inhibitors. It also blocks four earlier serine proteases in the blood-clotting cascade, limiting again its effective range of action. Even with all these controls, a small piece of a normal clot (an **embolus**) can break free and travel around the circulatory system, perhaps becoming lodged in a smaller blood vessel, where this unwanted blockage may become lethal. For example, if an embolism occurs in a coronary artery it can lead to localized cell death (myocardial infarction - MI). If it happens in the brain, it can result in a stroke, leading to nerve death and paralysis. An embolism in the lung can restrict the essential re-oxygenation of the blood. This has usually been blamed on cholesterol thickening the inner lining of blood vessels producing plaque that causes embolisms to more likely stick in the reduced 'piping'. In order to minimize the danger of embolism and to reduce the danger of thrombosis, as well as to clear away therapeutic blood clots formed during wound healing, the body introduces a new serine protease for dissolving clots called **plasmin**. This is a further, specialized member of the chymotrypsin family that attacks the collection of fibrin units making up a blood clot; these were designed to be dismantled. Like trypsin, plasmin cleaves protein chains after lysine-arginine subunits; there are over 360 such sites in fibrin, with the focus occurring on five primary sites that are all located in the thin connector arms of the fibrin molecule. Nature repeats its trick of making plasmin as an inert precursor, plasminogen that is activated by another enzyme, plasminogen activator (PA) or its popular name, "tissue plasminogen activator" ('**tPA**'). Its 700 amino acids are organized into several functional domains, especially the 250-amino acid serine protease domain that cleaves the arginine-valine linkage between subunits 560 and 561. The other larger part binds to a surface on the fibrin. When a clot is forming in an artery, there is generally a period of about 24 hours during which the open channel is being progressively being closed off by the growing clot; this is usually accompanied by growing chest pains as the heart muscle is suffering blood restrictions. Initially, live tPA was extracted (I am the direct beneficiary of this approach, as my earlier research had alerted me to this possibility, so I demanded this therapy from my cardiologists in the Emergency Room 20 years ago; **this saved my life**). Genentech were able to make this molecule using genetic techniques so that it is now very much cheaper. Sadly, too many heart surgeons are still inserting **stents** into people suffering MIs, as they make a lot more money from this than using a simple tPA injection. Statistics from American hospitals (gathered since 1990) have shown that this **tPA** approach can reduce death from 35% to under 9%. ** **READERS PAY ATTENTION** **

5.3 NERVOUS SYSTEM

An organism must know about its external surroundings and its internal context. It achieves these goals by the **nervous** system. In vertebrates, the nervous system divides into two parts: the central nervous system or CNS and the peripheral nervous system (PNS). The CNS is limited to the brain and the spinal column; the PNS consists of nerve bundles that link the CNS to every other part of the body. Nerves that transmit signals **from** the brain are called motor (or *efferent*) nerves, while those nerves that transmit information from the body **to** the CNS are called sensory (or *afferent*); [see *Organs*]. The brain exerts centralized control over all parts by fast electro-chemical signals and (more slowly) by directly controlling the secretion of chemicals called hormones. These two methods permit either fast or long-lasting, slower reactions to changes.

5.3.1 NEURONS

The nature of the nervous system was not discovered until 1900 when the structure of the neuron was finally investigated. The thousands of connections feeding input into a neuron are manifest as dendrites; they pick up chemical messages from nearby cells until a sufficient level is accumulated by the cell, when it 'fires' its message down its axon to its own terminii that discharge their message to the receiving dendrites. Each cell is prepared to play its role by a special enzyme in the cell's membrane, known as the **sodium-potassium** pump that completely spans the lipid membrane: its outer surface is in contact with the cell's environment and its inner surface with the cell's own cytoplasm. The function of the pump is to continuously import **potassium** (K^+) ions into the cell and export **sodium** (Na^+). In its initial conformation (E_1) it binds three sodium ions to specific sites on its internal (cytoplasmic) surface. The pump, as an enzyme reacts to these sodium ions by adding a phosphate group to the side chain of one of its own aspartic acid subunits drawn from an energetic adenosine triphosphate (ATP) molecule. This destabilizes the pump in its original conformation, so it is induced to undergo a change in shape to a new conformation, E_2 when the three sodium ions are brought to the outer surface, their attachment is weakened, so they are released in to the extracellular fluid. Now, two new sites are exposed on the outer surface that strongly bind 2 potassium ions as it then cleaves off its temporary phosphate group from the aspartic acid site. The pump reverts to its original conformation (E_1), relocating the K^+ ions to the inside of the cell where they are released into the cytoplasm. This then regenerates the site that binds the Na^+ ions, returning it to its original state, readying the pump for another cycle of action in milliseconds. The sodium-potassium pump is found in all body cells and its activity accounts for about 20% of the energy consumed by a cell. This enzyme is very prevalent in nerve cells and its activity may account for up to 70% of the energy consumed by them.

5.3.1.1 RESTING POTENTIAL

As the sodium-potassium pump transports three Na^+ ions out of the cell for every two K^+ ions transported in, an imbalance of electrical charge develops across the cell membrane. The inside of the neuron becomes electrically negative, while the outside becomes electrically positive; this separation of electrical charges is reflected in a voltage differential of – 0.07 volts (or –70 millivolts mV) across the cell membrane. It is this membrane potential (resting potential) that prepares the neuron for action.

5.3.1.2 FIRING THE NERVE

The actual 'firing' of the neuron (its transmission of a signal) results from a temporary disruption in the cell's resting potential. This is generated by another enzyme (ion channel) in the cell's membrane; these proteins open and close by a similar change in shape ('allosteric') caused by a fluctuation in the neuron's nearby resting potential. This shape change is caused by key electrically charged amino acids in the channel protein, which shift their orientation in response to the changed electrical forces. This leads to a change of Na^+ and K^+ ions between the inside and outside of the neuron: an electrical 'blip': the nerve-cell signal. The signalling begins with stimulatory changes that first develop in the neuron's dendrites and cell body. These result in a gradual erosion of the –70 mV voltage differential across the cell membrane. When this reduction reaches a threshold level, a critical event occurs. In the area of the axon adjacent to the cell body, the membrane channels for Na^+ suddenly undergo an allosteric shift in conformation in response to the change in the local electrical environment.

These "voltage-controlled" channels open and allow Na^+ ions (present in relatively high concentrations outside the nerve) to rush to the inside. This influx of positive charge leads to a complete collapse of the normal –70 mV resting potential in the proximal part of the axon. In fact, so many move across to develop a +20 mV positive state in this segment of the axon. This depolarization of the axon segment is a transient event, lasting only about one thousandth of a second, and is rapidly reversed. As soon as the depolarization has occurred the sodium channels spontaneously close and the membrane becomes locally resistant to the entry of further Na^+ ions. At the same time, a second set of channels opens fully and allows free passage of K^+ ions. This results in a strong outward flow of K^+ ions due to the high concentration of potassium inside the cell. As the K^+ ions leave, the positive charge inside the proximal segment of the axon begins to diminish until, after a few thousandths of a second, the original –70 mV resting potential across the membrane is restored. The two-stroke cycle of membrane depolarization (due to the opening of sodium channels and the influx of Na^+) and membrane repolarization (due to the secondary opening of potassium channels and the diffusion of K^+ back out of the cell) is called an **Action Potential** (the change in electrical potential associated with nerve action). It is this spread of activity down and along an axon to the end of the axon, where the wave of depolarization reaches the axon terminal opening a voltage-controlled Ca++ channel, facilitating neurotransmitter release.

5.3.1.3 NEURO-TRANSMITTERS

The general solution to the problem of neurotransmitters was first solved by the German physiologist Otto Löwi in 1921 that was rewarded with the Nobel prize in 1936. Here, he identified the chemical released by the vagus nerve (as it controlled the heart beat) as ***acetylcholine*** ($C_7H_{16}NO_3$). This small molecule is now known to be the transmitting agent between many neurons, in organisms ranging across a broad spectrum: from insects to higher vertebrates. It is also the neurotransmitter between nerve cells and muscle cells.

The intricate structure of the nervous system is made even more complex by the existence of a multiplicity of neurotransmitters, linking the trillion (10^{12}) neurons to each other and to other target cells through at least one hundred trillion (10^{14}) synaptic connections. More than 50 neurotransmitters are now known, ranging in size from small molecules to peptides with as many as 30 amino acids. Some exert a stimulatory influence on their target cells (causing depolarization), others an inhibitory influence (hyper-polarizing the target cell); some are easily released, others only upon repeated axon depolarization; some are short-lived, others more persistent. As a result of this variety of neurotransmitters, the flexibility and sophistication of the nervous system is dramatically increased.

5.3.1.4 CROSSING THE SYNAPSE

The location where the neurotransmitters work is the **synapse**: the junction between a neuron and its target cell (either the next dendrite in a neural network **OR** the surface of a muscle cell). The membranes of the two adjacent cells are separated by a tiny, specialized intercellular space filled with complex proteins and carbohydrates that form a connective matrix. Neurotransmitters are synthesized near the end of the axon by enzymes present in the cell cytoplasm. The synthesis of **acetylcholine** is formed from two simpler compounds: an acetic acid component (derived from glucose) plus a 10 atom molecule of choline (usually attached to lipids in the cell membrane), that is boosted by the enzyme Choline AcetylTransferase (CAT) that only exists in nerve cells. When the wave of depolarization reaches the axon tip, it opens special calcium channels, which permit the entry of Ca^{++} ions into the neuron; these promote the fusion of synaptic vesicles (containing the neurotransmitters) with the cell membrane discharging thousands of neuro-transmitter molecules into the synaptic cleft as they move towards the target cell.

5.3.1.5 RECEIVING THE SIGNAL

The neurotransmitter molecules drift across the cleft, where they are captured by the thousands of specialized proteins in the membranes of the post-synaptic cell through an enzymatic lock-and-key method. In the well-studied case of the neuro-muscular junction, the arrival of acetylcholine in the receptor triggers an (allosteric) shape-change so that it opens and becomes a pump for a variety of positively charged ions.

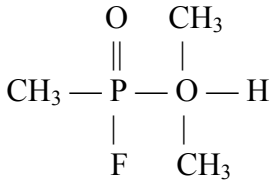
The neurotransmitter receptor becomes the fourth of the major nervous system-specific enzymes needed. Each receiving dendrite has its voltage changed a little by each neurotransmitter molecule processed; these are added all together and if the receiving cell's membrane potential falls below -20 mV it will trigger the 'firing' of its axon too. This is the trick of combining 'analog' actions and 'digital' reactions. The toxin of the cobra snake, like the Amazonian poison curare, functions by blocking the acetylcholine receptor, thereby inducing paralysis at neuromuscular junctions that may be fatal.

5.3.1.6 TURNING OFF THE SIGNAL

This brings us to the last (the fifth) enzyme needed for the neurological system: a neurotransmitter processing enzyme that switches off excess acetylcholine, called **acetyl-cholinesterase**, which cleaves the acetylcholine back to its original molecules (acetic acid and choline).

5.3.2 NERVE GASES

Doctors accidentally discovered the threat of blocking acetyl-cholinesterase; this led to the deliberate making of Nerve Gases, like **sarin**: one of the most lethal weapons of mass destruction ever discovered. These tiny molecules, easy to mass produce in a chemistry laboratory anywhere from reagents generally available to industrial chemists. It is lethal in tiny doses (1 milligram: about one small shake of salt). Nerve gases enter the body by inhalation or direct absorption through the skin, spreading through the body until they reach any nerve, where they induce death within minutes by blocking the ability to breathe, resulting in asphyxia. Even in small doses, they are still lethal after several hours, as the damage caused is cumulative and irreversible. These are part of the major chemical family known as organophosphates that arose from agricultural research; ironically, these are related to the 'Star' of this story: chymotrypsin as the di-isopropyl-phosphofluoridate (**DPF**) binds irreversibly to the Serine-195, as the crucial amino acid at the center of the enzyme's reaction mechanism. Nerve gases work in exactly the same way as DPF, except that they are much more effective in blocking the active-site serine. Their more powerful effect comes from their greater reactivity and greater stability, once attached to the enzyme. At the inter-neuronal and neuro-muscular junctions they affect, these compounds cause the buildup of acetylcholine and the over-stimulation of target cells. The diaphragm is one of the muscles that is innervated by nerves that use excess acetylcholine. The chemical simplicity of **sarin** is frightening:



SARIN

Sarin has the same volatility as water, so it would be released as a vapor cloud. Nerve gases were discovered by Nazi scientists but Hitler refused to use them, perhaps remembering his own personal exposure to the much simpler mustard gas in World War I but more likely as he assumed (incorrectly) that the Allies had similar weapons and would retaliate in kind. Both the US and the Soviets have large stockpiles of nerve gases and they plan to use them in battlefield operations before they use nuclear bombs. The only thing protecting the public from these obscene weapons is a piece of paper: the prohibition agreed to in Geneva banning their use in 1925 but several nations (including USA, UK, Russia, France and China) interpret this as 'No-First-Use' and reserve the right to retaliate in kind, if such weapons are used against them. Such is the Madness of the Human Race, as they pursue all kinds of weapons research in the name of science. Meantime, better gas masks and protective clothing have been issued to troops everywhere but they must be used immediately, so more money has been invested in faster Nerve-Gas Detectors.

Living cells contain thousands of enzymes, many of them operating at the same time; their catalytic actions generate a complex network of metabolic **pathways**, each composed of sequences of chemical reactions, in which the product of one enzyme becomes the substrate of the next. There are usually many branch-points (nodes) where different enzymes compete for the same substrate. This is so complex that elaborate controls are required to regulate when and how rapidly each reaction occurs.

Negative-feedback is a normal method of enzyme activity regulation of reaction rates; using a direct, reversible change in response to the presence of specific small molecules especially when a product created late in a pathway inhibits an enzyme earlier in the pathway. Positive-regulation occurs when a subsequent product in one branch of the network then stimulates the activity the activity of an enzyme in another pathway. Another regulatory method (often used in eukaryotic cells) is the addition of a **phosphate** group to one of the protein's amino acid side chains that can cause a major conformational (shape) change in the protein; each of these phosphorylation events requires one molecule of ATP; conversely, removing the phosphate has an equally dramatic effect.

5.3.3 ENKEPHALINS

Morphine disrupts a normal nerve pathway so that it can suppress pain, allowing it to play a major role in medicine for centuries. It is used in the most serious of medical illnesses and no better pain-suppressing agent has been found in one hundred years of research. Morphine is a major part of the seeds of the opium poppy (accounting for up to 10% of the weight of the plant). The simple addition of two acetyl groups (CH_3CO_2) generates the related molecule, Heroin which is soluble in lipid membranes, enters the brain more easily and is more effective. Both molecules are closely related to the *enkaphalins*: the amino-ends of a pair of short peptides: Tyr - Gly - Gly - Phe -{Met **or** Leu}.

These molecules are produced by certain neurons in the brain at times of great exertion or stress. They are used as inhibiting neurotransmitters intercepting nerve signals that would otherwise be perceived as pain. They do this by the **enkaphalins** interacting with the receptors (membrane ion channels) in the target neurons so as to make their depolarizations more difficult. The enkaphalins are derived from a large 'polyprotein' containing about 100 amino acids that contains about eight enkaphalin units at various places, with each five-amino unit being bounded by a pair of either lysine or arginine subunits. The ability of morphine or heroin to mimic the physiological effect of the enkaphalins is due to a specific aspect of their structure. One surface of the morphine molecule is like the N-terminal **tyr**osine subunit that occurs in both enkaphalins. It is this similarity in structure that allows morphine (after it has diffused into the synaptic space between brain neurons) to trigger the same response as the enkaphalins and mitigate anxiety and pain. However, in the body, he normal enkaphalins are rapidly degraded by proteolytic digestion after their use as neurotransmitters; morphine and heroin (because of their different structure) remain intact. Their effect is therefore more prolonged and severe and can lead to death.

5.3.4 EMOTIONS

The lethal effects of high concentrations of chemicals that inhibit acetyl-cholinesterase are well known but what might be the effects of sub-lethal exposures? These effect our behavior, with results seen as a violent change in activity and higher levels of cortical control. Author Dressler recounts the story of an unlucky gardener in 1983 who was exposed to a lawn care insecticide that used a chemical like DPF [§5.3.2]. This caused him to attack his client and murder her. Even though two expert-witness toxicologist were called, the jury still convicted him because others exposed to this product did not murder anyone. This exposes the failure of medical science to convince the public of the highly individual responses to chemicals.

6 SUMMARY

This essay has attempted to provide insights into the greatest revolution in science in the last 150 years. In 1850, the science world was dominated by the simple views of nature reflected in the mechanical models of Newton's physics and the simple atomic models of Dalton's chemistry. This was when a few chemists began investigating the molecular substances found in living systems; initiating the study of the complex world of biochemistry. They first investigated proteins, uncovering their simpler units of amino acids. It was not long before the incredibly complicated world of enzymes surfaced that showed how proteins grew in complexity as they enfolded upon themselves and interacted in bizarre ways to account for all the physiological processes needed to keep large organisms alive. They realized that these molecular machines **interacted in vast networks of activity** through a series well-defined chemical transformations.

They realized that these superbly organized, twisted collections of only 20 amino acids could produce astonishing results: their catalytic power multiplied the speed of chemistry from years to milliseconds to keep it all working well together. As well as their speed, enzymes possess an astonishing ability to promote specific interactions.

Dressler here has done a fine job of describing just a few of the physiological actions of a single enzyme used in the body. Beginning with digestion, this master enzyme, *Chymotrypsin*, has evolved to help with vital processes throughout the body to include blood-clotting and signal transmission in the nervous system where it was seen that trans-membrane enzymes acted like pumps; the electrical effects were slowed down to electrochemical messages that allowed the brain time to process and react in an organized manner. As messages reached the end of a neuron, they released neurotransmitters to cross the gap to the next cell. This effect had to be switched off before the chemicals accumulated to dangerous levels; this failure has been exploited by the mad men who run our nations to produce deadly **nerve gases** or even dangerous **insecticides**.

In this 'wrap-up', I shall try to bring together the positive highlights (successes) of what has been uncovered in the last one hundred years but also try to show what tough lessons have been learned and the major challenges that still must be overcome.

For example, the complex use of chemical enzymes in neuronal transmissions are critical to allow for time delays to complete the activity: these **CANNOT** be replicated in electrical digital circuits for A.I. This story has also hopefully conveyed the vast levels of **complexity** in keeping a living organism alive. The many correlated actions (as in blood-clotting §5.2) seem far too complex to have evolved in a simple linear manner; this throws more cold-water on the simplistic, verbalizations that are casually thrown around by biologists to justify Darwin's model of Random-Change as a mechanism for generating Evolution.

In other words, there is a huge **GAP** in knowing a biological structure and **how** the information is used: even in a single cell. In organisms like humans, with over 200 cell types, there is then the further challenge of finding all the linkages, including messenger molecules, between all the cell types that trigger new processes in the remote cells. Tragically, human beings (including scientists) have little experience throughout all of human history in untangling complexity and with living cells, we have uncovered the most complexity possible. Our **short-term** thinking encourages us to release a chemical into our world to achieve one possible beneficial result (for profit); only later discovering some dreadful, unexpected side effects. A level of experimentation on uninformed people was deemed a war-crime in 1945. As we have been told that "mRNA is the future vaccination strategy" it is hoped that this paper will help educate more people to minimize personal risk when advised to "follow the crowd". Think about the Unknowns!

6.1 REVIEW ALERT

Note: a large 55-page section has been omitted here {from page # 96 through page# 151} as it involves many diagrams and is better suited to another essay on chemical bonding. Another large 41-page section has been also omitted here {page# 153 through page # 194}as this focused on vague theories of the general enzymatic actions of one specific enzyme (*Chymotrypsin*) by analyzing just (2) of the active sites in this molecule with its over 4,000 atoms; focusing on only two of the 250 amino acids (histidine at position 57 and serine at 195), for weak theories in molecular evolution. Readers may consult Dressler directly if interested in the omitted areas.